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TITLE: Cortical Lesions as Determinants of White Matter Lesion Formation
and Cognitive Abnormalities in MS

PRINCIPAL INVESTIGATOR: John D. Port, M.D., Ph.D.

CONTRACTING ORGANIZATION: Mayo Clinic
Rochester, MN 55905

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14. ABSTRACT This study evaluates for the presence of connectivity between cortical lesions and white matter lesions in patients with MS. During the report period, we have statistically documented this connectivity, presented our findings at 2 national meetings, and have submitted a paper to Neurology. Using one of our novel imaging sequences, we have documented a characteristic "halo" around larger MS lesions that seems specific to MS. We are currently collecting and analyzing data from our positive control cohort to confirm that this finding is not noted with other types of white matter pathology. Both lesion connectivity and MS lesion halos may serve as novel new imaging biomarkers for the disease. We have explored automated cortical lesion detection. We have begun preparation for tract-based and normal-appearing white matter analysis using DTI derived metrics as well as image intensity ratios.					
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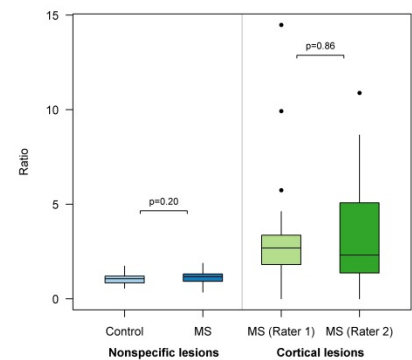
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1. **INTRODUCTION:** Multiple sclerosis is an idiopathic inflammatory disease with the pathological hallmark of demyelinating white matter lesions (WMLs). Over the last 2 decades, studies have unequivocally confirmed that there is also clinically significant cortical gray matter involvement by characteristic cortical lesions (CLs). Our recent animal work supports the hypothesis that WMLs develop in areas connected with CLs via projecting pathways from gray matter. Our pilot data from MR imaging studies of MS patients suggests that CL-WML connectivity exists and is present in early MS. The purpose of this study is to develop and use a novel gray matter double inversion recovery (GM-DIR) MRI sequence to better detect and characterize CLs and their connectivity. We propose 2 specific aims: 1) to study the connectivity of CLs and WMLs using a combination of sequences (including the new GM-DIR sequence), cortical thickness measurement, and DTI-based probabilistic tractography; 2) to assess neurocognitive performance in the study cohort using a standardized cognitive battery and determine potential correlations with the proposed MRI metrics of cortical damage. The scope of this research involves performing a prospective cross-sectional case-control study of 100 early MS patients, 30 positive controls with white matter findings on imaging but no MS, and 20 healthy controls with no white matter findings on imaging. The MS subjects will also undergo an assessment of neurocognitive performance using the MACFIMS battery. CL and WML connectivity analysis will be performed, as well as lesion characterization. Lesion connectivity and characterization data will be correlated with cognitive deficits on the MACFIMS.

2. **KEYWORDS:** multiple sclerosis, MRI, MACFIMS, white matter lesion, gray matter lesion, diffusion tensor imaging, double inversion recovery imaging, connectivity

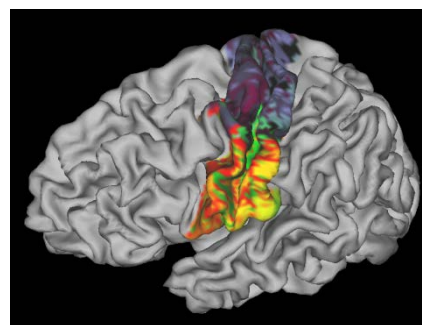
3. **ACCOMPLISHMENTS:**
 - **What were the major goals of the project?**
 - Subject recruitment: Recruiting has been slow: we have enrolled 45/100 subjects with MS, 14/30 positive control subjects, and 16/20 healthy control subjects. Almost all of our MS subjects have completed the MACFIMS neurocognitive testing.
 - MRI acquisition: processes for image acquisition, storage and pre-processing were completed by approximately June, 2014. These processes are adjusted as necessary to insure subject safety and accommodate minor changes in analysis technique.
 - Neuropsychological testing: Data acquisition is ongoing for new MS subjects. To date we have MACFIMS data on the majority of our subjects, with a few incomplete due to subject drop-out.

- **MRI data processing:** Pre-processing pipelines were completed by approximately June, 2014. Post-processing pipelines are completed and ready for data analysis. We have optimized our data analysis technique with minor revisions and continue to implement quality control and additional optimizations. We recently completed programming of our lesion detection and scoring tool.
- **Post-processing pipelines** are completed and ready for data. We have recently completed programming of our lesion detection and scoring tool.
- **Statistical Analysis:** Statistical analysis pipelines are in place. Once lesion scoring and connectivity analysis are complete, data will be statistically analyzed.
- **What was accomplished under these goals?**
 - Regarding lesion connectivity, we have successfully documented connectivity of cortical lesions to deep white matter lesions in MS. The box plot at the right summarizes the results of the study. Briefly, two independent raters (Raters 1 and 2) reviewed all subjects and identified cortical and white matter lesions. Diffusion tensor data was used to draw tracts from each CL to each WML and vice versa. A cortical lesion ratio (CLR) was calculated as the ratio of the volume of CLs in connected cortex/total volume of connected cortex over the volume of CLs in non-connected cortex/total volume of non-connected cortex. The box plot shows the CLR for nonspecific lesions in controls and MS patients as well as for CL detected by both raters. Note that the CLR is significantly increased for both raters (Rater1=2.7, Rater2=2.3) compared to normal controls (CLR=1.0) as well as synthetic lesions placed in MS patients (CLR=1.2). These findings document significant connectivity in the live MS patient of CLs to WMLs, possibly representing a new imaging biomarker for MS. This work was presented at the ACTRIMS-ECTRIMS and AAN annual meetings, and we have submitted the paper to Neurology (currently under review)
 - Regarding lesion characterization, our novel GM-DIR sequence has yielded a potentially valuable finding. Specifically, larger MS lesions (more than 7-8 mm in diameter) demonstrate a low signal “halo” around a high signal “interior” (see figure). This finding has been previously noted on scans obtained using 7T MRI scanners, but we are the first to show the finding using a clinical 3T MRI scanner. If this finding is specific to MS, this could represent a new imaging biomarker for the disease, greatly aiding in the diagnosis. We have accelerated the recruitment of our positive



control subjects, and to date have not observed the halo in subjects with stroke, edema or leukoaraiosis. Once we have completed recruiting our N=30 positive control subjects and can verify that the halo only occurs in MS patients, we will publish a paper based on this finding.

- Regarding lesion detection, we have currently finished developing a lesion rating tool and will begin scoring all MS lesions on our available acquired data using this tool. As an exploratory aim, we have worked on the development of an automated cortical lesion detector. Briefly, all image volumes are co-registered using boundary-based registration and are checked visually for accuracy. The cortex and immediate subcortical white matter are divided into four layers (outer cortex, middle cortex, inner cortex, subcortical white matter) using Freesurfer. The image intensity from the 4 different sequences for each of the 4 cortical layer plus the cortical thickness are extracted as a 17-element vector, and the affinity propagation algorithm is used to cluster similar cortical regions with the hope that MS lesions would cluster together. The figure on the right demonstrates the 117 clusters extracted from 4023 vectors for the pre- and post-central gyri; note how some of the Brodmann's areas have become visible. This tool needs further work, but may become more reliable than human raters and allow for automated lesion detection, especially when the entire cortex can be analyzed simultaneously.



- **What opportunities for training and professional development has the project provided?**
 - Nothing to report.
- **How were the results disseminated to communities of interest?**
 - Nothing to report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - Subject recruitment: Ongoing. Hire an additional study coordinator to help finish recruiting all subjects.

- MRI acquisition: complete.
- Neuropsychological testing: Ongoing. Continue to gather MACFIMS data from MS subjects.
- MRI data processing: We have enlisted the help of two additional neuroradiologists to assist with lesion detection and scoring, and will begin scoring lesions on all existing data in June 2015. Once lesion scoring is completed, connectivity analysis will begin via our automated post-processing pipeline. GM connectivity will be assessed, as well as analysis of DTI-based scalar parameters to search for findings in normal-appearing white matter.
- Statistical Analysis: Once lesion scoring and connectivity analysis are complete, data will be statistically analyzed.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
 - Nothing to report.
- **What was the impact on other disciplines?**
 - Nothing to report.
- **What was the impact on technology transfer?**
 - Nothing to report.
- **What was the impact on society beyond science and technology?**
 - Nothing to report.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
 - Dr. Tillema had recently received a K-award from the NIH and no longer had sufficient effort available for this study. Dr. Atanga was cross-trained on lesion scoring techniques, and we requested that he replace Dr. Tillema on the project. This change was reviewed and approved in modification P00001 for W81XWH-13-1-0098 dated 25-Jun-2014.
 - Dr. Pirko had been ill for several months before he died on November 30, 2014. As a result of his death, we requested that the PI was changed to Dr. Port, one of the co-investigators on the study. Furthermore, due primarily to his extended illness, recruiting is significantly delayed and a 1-year no-cost extension for the study was requested. These changes were reviewed and approved in modification P00002 for W81XWH-13-1-0098 dated 4-Mar-2015.
- **Actual or anticipated problems or delays and actions or plans to resolve them**

- As a result of Dr. Pirko's illness and subsequent death, enrollment slowed significantly during that period. Furthermore, his initial estimates of the number of patient meeting inclusion criteria were optimistic; while we see many MS patients each week, few turn out to be early RRMS or CIS eligible under the inclusion criteria, or have a busy agenda that prohibits them from participating. Recruiting age-matched positive controls has also been difficult, as white matter and cortical pathology in younger patients is relatively rare.

To remedy this situation, we plan in implementing the following plans:

- For MS patients: All patients with appointments scheduled 2 months out are pre-screened for eligibility and contacted in advance if they meet inclusion criteria. Each week, all appointments are pre-screened for eligibility, capturing any patients added onto the schedule since the 2-month look-ahead. Dr. Tillea personally contacts the neurologist on the MS service each Monday and Wednesday searching for eligible patients. This captures any newly-diagnosed patients.
- For Positive Control subjects: We have implemented a computer search of all head MRI reports from the previous day. Any exams which contain selected key words (e.g., "moderate leukoaraiosis") are flagged and reviewed Drs. Tillema and Port for eligibility and inclusion. Dr. Port has also enlisted the help of other neuroradiologists to identify patients that may be eligible for inclusion as positive controls.
- For Healthy Control subjects: We have a word-of-mouth system to identify and recruit healthy controls subjects. This system works very well and we are ahead of plan for our normal controls.
- We are hiring additional study coordinator support to aid our recruiting efforts.
- **Changes that had a significant impact on expenditures**
 - Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
- **Journal publications.**
 - Tillema JM, Weigand S, Port JD, Mandrekar J, Shu Y, Lucchinetti CF, Pirko I. In vivo Detection of Connectivity Between Cortical and White Matter Lesions in Early MS. Submitted to Neurology
- **Books or other non-periodical, one-time publications.**
 - Nothing to report
- **Other publications, conference papers, and presentations.**

- Tillema JM, Port JD, Weigand S, Mandrekar H, Shu Y, Lucchinetti CF, Pirko I. MRI Reveals Connectivity of Cortical Lesions to Deep White Matter Lesions in Multiple Sclerosis. 2014 Joint ACTRIMS-ECTRIMS Meeting, Boston, September, 2014.
- Tillema JM, Weigand S, Mandrekar J, Shu Y, Lucchinetti CF, Pirko I, Port JD. MRI Reveals Connectivity of Cortical Lesions to Deep White Matter Lesions in Multiple Sclerosis. 67th American Academy of Neurology Annual Meeting, Washington DC, April, 2015.
- **Website(s) or other Internet site(s)**
 - Nothing to report
- **Technologies or techniques**
 - Nothing to report
- **Inventions, patent applications, and/or licenses**
 - We have filed a patent application entitled “System and Method for Producing Imaging Biomarkers Indicative of a Neurological Disease State Using Gray Matter Suppression via Double Inversion-Recovery Magnetic Resonance Imaging.” Patent application #: 61/880,386. Patent type: provisional.
- **Other Products**
 - Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**
 - No change
- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Yes. Dr. Pirko has died, so his effort has decreased from 20% time to 0% time.
 - Nothing else to report.
- **What other organizations were involved as partners?**
 - Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.

9. APPENDICES: n/a